Mycorrhiza – Carbohydrate and Energy Metabolism

RÜDIGER HAMPP and CHRISTOPH SCHAEFFER[1]

1 Introduction

The roots of most trees and shrubs and many herbaceous plants are invaded by mycorrhizal fungi. The most common of these symbiotic associations in trees of temperate forests are ectotrophic. In this type, hyphae extending from a mycelial layer covering the surface of fine roots penetrate between root cells and form characteristic structures (called Hartig net) in the outer cortex. Some trees and many terrestrial flowering plants develop arbuscular mycorrhizas (AM). Estimates are that up to 90% of angiosperms establish this kind of symbiosis (e.g. Law 1985). Here, no superficial mycelial layers nor specific morphological extracellular structures such as the Hartig net are formed. Because of the different kinds of intrusions into the host cells formed by fungal membranes, this type is also called vesicular-arbuscular mycorrhiza (VAM). The development of these symbiotic structures is a complex process which depends on a variety of internal and external factors (e.g. Harley and Smith 1983) of which mineral nutrition and the supply of carbohydrates by the host plant are most important. On the background of the excellent review on mycorrhizal properties published by Harley and Smith in 1983, we focus on literature appearing since then and try to evaluate former conclusions on the basis of recent data. The reader is also referred to other reviews on carbon metabolism in mycorrhizas (Cooper 1984; Lewis 1986; Harris and Paul 1987; Martin et al. 1987; Jakobsen 1991; Schwab et al. 1991; Finlay and Söderström 1992; Smith and Read 1996).

[1] Universität Tübingen, Physiologische Ökologie der Pflanzen, Auf der Morgenstelle 1, D-72076 Tübingen, FRG

[2] **Abbreviations:** AM, arbuscular mycorrhiza; ECM, ectomycorrhiza; F26BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; P_i, inorganic phosphate; TP, triose phosphates; VAM, vesicular-arbuscular mycorrhiza

A. Varma, B. Hock (Eds.), Mycorrhiza, 2nd Ed.

2 Carbohydrate Metabolism

2.1 Fungal Carbohydrates

Polyols such as glycerol, mannitol, and arabitol, in addition to trehalose and glycogen have been detected in mycorrhizas (Bevege et al. 1975) and were labelled after feeding individual host plants with $^{14}CO_2$ (Söderström et al. 1988). ^{13}C-NMR spectroscopy applied on intact mycelia from different mycorrhizal fungi revealed glycerol, mannitol (*Cenococcum graniforme*), trehalose (*Laccaria proxima*), and glycogen (*Hebeloma crustuliniforme*) as the main carbohydrates at the end of the log phase of growth (Martin et al. 1984).

Trehalose, a non-reducing disaccharide made up of glucose, is a carbohydrate preferably synthesized by fungi, although there are reports on its presence in some spermatophytes (Hopf and Kandler 1976), in pteridophytes (e.g. Adams et al. 1990) and algae (e.g. Avigad 1982). Organs of higher plants that contain trehalose are most commonly involved in microorganismic interactions such as nitrogen-fixing root nodules of *Alnus* (Lopez and Torrey 1985) or legumes (Salminen and Streeter 1986), or mycorrhizas (Harley and Smith 1983). In AM roots, e.g., of *Tagetes tenuifolia* and *Glycine max* trehalose was present but absent in non-mycorrhizal roots (Schubert et al. 1992). Similar observations exist for ECM (Hampp et al. 1995).

This interaction-related presence of trehalose is of special interest as trehalose can possibly act as an inhibitor upon plant metabolism, although no specific effects have been reported yet (Veluthambi et al. 1981; Mellor 1992). Thus, it is suggested that only plant tissues able to detoxify trehalose are able to symbiotically interact with the respective microorganisms (Mellor 1992). Trehalase has been shown to be present in cell cultures of conifers (Kendall et al. 1990) and radicles of spruce (Wallenda 1996), and for soybean root nodules a plant-specific trehalase has been characterized recently (Müller et al. 1992).

Trehalose was the principal sugar formed in excised mycorrhizas of spruce trees (Niederer et al. 1989). Its content increased concurrently with the degree of fungal infection, and decreased upon P_i fertilization and light deprivation which also caused a decrease in mycorrhization. Thus trehalose can be taken as marker for the degree of mycorrhization of fine roots (similar to ergosterol or chitin). It should be noted, however, that in contrast to the latter two compounds, trehalose levels can vary much more as they depend considerably on the availability of host-derived photoassimilates (Wallenda et al. 1996).

According to Lewis and Smith (1967), D-mannitol is the most abundant sugar alcohol in fungi. Its function is discussed to be of dual nature, i.e. a storage pool for carbohydrate and protection against temperature (Pons et al. 1986) and drought stress (Lewis and Smith 1967). With regards to the symbiotic interaction it is important to know that root cells are obviously not able to significantly metabolize mannitol (Lewis and Harley 1965c; Jirjis et al. 1986). As with trehalose, the other fungus-specific carbohydrate (see below), this enables the fungal partner to build up a carbohydrate gradient by withdrawing

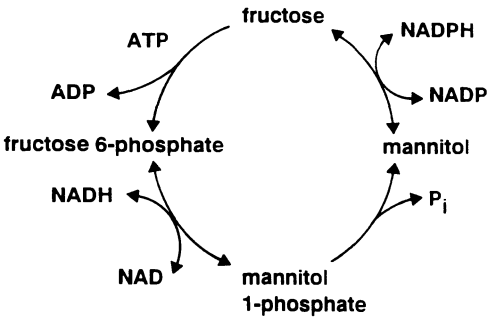

Fig. 1. Mannitol cycle as suggested by Hult et al. (1980)

plant carbohydrates and converting them into products which cannot be accessed nor metabolized by the host cells.

2.2 Metabolism of Mannitol and Trehalose

As suggested for the deuteromycete *Alternaria alternata* (Hult and Gatenbeck 1978) synthesis and degradation of mannitol are performed in a cyclic manner. Enzymes involved are hexokinase, (NAD) mannitol 1-phosphate dehydrogenase, and (NADP) mannitol dehydrogenase (Fig. 1; see also Hult et al. 1980). However, these enzymes are not generally present in mycorrhiza-forming fungi. In addition to deuteromycetes, this so-called mannitol cycle has also been verified in the mycorrhizal ascomycete *Sphaerosporella brunnea* but not yet in basidiomycetes (Ramstedt et al. 1987; see also Table 1). Although mannitol can be detected in basidiomycetes, its synthesis is still unclear (Ramstedt 1988; Jensen et al. 1991). By applying ^{13}C-NMR techniques, Martin et al. (1985, 1988) could show that some of the glucose taken up by the ascomycetes *Cenococcum graniforme* and *Sphaerosporella brunnea* is transformed into trehalose after cycling through the mannitol pool. The authors therefore suggested that the mannitol cycle should be of importance in the regulation of glucose metabolism and the formation of storage carbohydrates (Martin et al. 1988).

The biosynthesis of trehalose is analogous to that of sucrose. Trehalose 6-phosphate is formed from UDP-glucose and glucose 6-phosphate via trehalose 6-phosphate synthase (Martin et al. 1987). Hydrolysis of trehalose is by a specific α-glucosidase (trehalase: Thevelein 1984).

According to the degree of accumulation of mannitol and trehalose, ectomycorrhizal fungi can be distinguished. Obviously, ascomycetes preferably accumulate mannitol while it is trehalose which is primarily stored in basidiomycetes (cf, Niederer 1989). This also has consequences for the preferred source, carbohydrate. According to Lewis and Harley (1965a,b), the

Table 1. Occurrence in fungi of enzymes involved in mannitol metabolism. The data are taken from Ramstedt et al. (1987) and compiled as in Wingler (1992)

	Mannitol 1-phosphate dehydrogenase	Mannitol 1-phosphatase	Mannitol dehydrogenase
Ascomycetes	NAD-dependent	Substrate specific	NADP-dependent
Basidiomycetes (mycorrhiza-forming)	Not detected	Substrate specific	NADP-dependent
Basidiomycetes (non-mycorrhiza-forming)	Not detected	Unspecific	NAD-dependent

synthesis of trehalose (in mycorrhizas) and of glycogen relies on glucose, while fructose is mainly converted into mannitol. This should certainly have consequences on the supply of carbohydrates by the host tissue with respect to the type of fungal partner (see I. Jakobsen, this Vol.).

2.3 Carbon Requirement for the Growth of Mycorrhizal Fungi

Carbohydrate dependency of mycorrhizal fungi has been tested by their ability to grow on media containing specific carbon sources. Owing to the lack of cultivation methods for AM forming fungi, this approach has up to now only been possible with those species involved in ericoid mycorrhizas and ectomycorrhizas (ECM) (for reviews, see Jakobsen 1991; Williams 1992). Major carbon sources are glucose and fructose (Jennings 1995). An exogenous supply of glucose has been shown to support ectomycorrhizal infection in a range of species (Theodorou and Reddell 1991). There are, however, also reports that mannose, sucrose, and raffinose can substantially support mycelial development of *Tuber melanosporum* (Mamoun and Olivier 1991), *Cenococcum geophilum*, *Rhizopogon roseolus*, and *Suillus bovinus* (Giltrap and Lewis 1981). Evidence for utilization of sucrose also comes from feeding glucose- and fructose-labelled sucrose to isolates of *Pisolithus tinctorius* (Taber and Taber 1987). From the products of hydrolysis glucose, in contrast to fructose, was readily oxidized to CO_2. According to this observation, invertase activity should be present in the fungal material. It is, however, quite possible that, due to the low medium pH, sucrose was hydrolyzed non-enzymatically. Sucrose hydrolysis is significant at pH values below 4. In the experiment of Giltrap and Lewis (1981), sucrose containing media were not buffered and the pH was below 3 (their Table 1). This view is supported by reports which show that ECM-forming fungi such as *R. roseolus* cannot make use of sucrose without the addition of glucose (Palmer and Hacskaylo 1970; Lamb 1974). Uptake of glucose will cause acidification of the (unbuffered)

medium which then will favour sucrose hydrolysis. Further evidence for this scenario comes from experiments with other ECM fungi. *Amanita muscaria* or *Hebeloma crustuliniforme* can obviously make no direct use of sucrose, while glucose and fructose are readily consumed (Salzer and Hager 1991). Apoplast invertase located in the host cell wall appears to be the only physiological way to make sucrose available for these fungi during symbiotic interaction. Although important for assimilate transfer, the activity of acid invertase in the system *Picea abies/Amanita muscaria* is not related to the degree of fungal infection (Schaeffer et al. 1995). Rates of sucrose hydrolysis in non-mycorrhizal roots are, however, obviously high enough to support symbiotic requirements (Schaeffer et al. 1995).

Mycorrhizal fungi are also able to degrade complex organic structures such as lignin or others (Abuzinadah and Read 1986; Haselwandter et al. 1990; Jennings 1995) or assimilate CO_2 via carboxylases (Wingler et al. 1996).

2.4 Host Carbohydrate Metabolism

2.4.1 Carbohydrate Pools

According to Wedding and Harley (1976), mannitol could interact with enzymes of the host carbohydrate metabolism. In their experiments with beech mycorrhizas, mannitol and also threitol were claimed to inhibit glucose 6-phosphate dehydrogenase, phosphofructokinase, hexokinase, aldolase, and phosphoglucomutase. This finding was discussed as a possible way of manipulating host carbohydrate metabolism by the fungal partner. This suggestion, however, has not been verified yet. Trials to repeat such effects with enzyme preparations from beech, pine (Jirjis et al. 1986), or spruce rootlets (H. Thelen, pers. comm.) were unsuccessful.

Inspite of this lack of evidence for direct interaction by a certain metabolite, there is some impact of mycorrhization on carbohydrate pools in the host. *Fraxinus* seedlings inoculated with *Glomus macrocarpum* were lower in starch and soluble sugars than non-mycorrhizal seedlings (Borges and Chaney 1989). Soybean plants inoculated with *Glomus mosseae* did not show differences in leaf sugar concentration but had higher starch contents in comparison to uninoculated controls. Starch decreased, however, with the length of mycorrhizal infection (Brown and Bethlenfalvay 1986). In contrast, soybean inoculated with *Glomus fasciculatum* was shown to contain less starch than corresponding P_i-fertilized (non-inoculated) controls (Pacovsky 1989a).

Infection of oak seedlings with *Pisolithus tinctorius* was significantly correlated with the fructose content of short roots (Dixon et al. 1981). This relationship was less obvious for extracts of *Glomus mosseae*-infected wheat roots from some cultivars which had increased amounts of total and reducing sugars but no clear relationship between sugar content and the degree of mycorrhization (Ocampo and Azcón 1985). With other cultivars the same

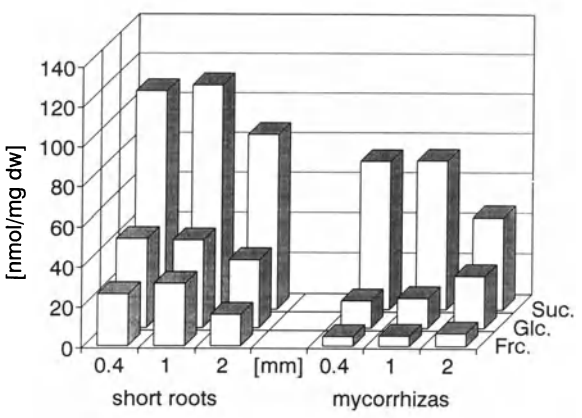

Fig. 2. Amounts of soluble carbohydrates contained in short roots of spruce, non-mycorrhizal or mycorrhizal with *Amanita muscaria*. Roots are grouped according to length (0.4 to 2 mm). S.D. (n = 6) between 0.5 and 15% of mean value. Suc. = sucrose, Glc. = glucose, Frc. = fructose

authors reported that the infection led to a decrease in the reducing and total sugar content, which in this case was correlated with the degree of mycorrhizal infection (Azcón and Ocampo 1981). This is in accordance with results on mycorrhizal (*A. muscaria*) roots of spruce seedlings. The total amount of sucrose + glucose + fructose was about 30% higher in non-mycorrhizal fine roots, sucrose being the dominating sugar in both mycorrhizal and non-mycorrhizal fine roots (Fig. 2; see also Rieger et al. 1992). Similarly, Wallander and Nylund (1991) found lower levels of these sugars in mycorrhizal roots of *Pinus sylvestris* when compared to non-mycorrhizal controls.

In contrast, root exudates of a range of plant species infected with *Glomus mosseae* showed no relationship between total sugar content and AM formation (Azcón and Ocampo 1984). In *Citrus/Glomus fasciculatum* associations the level of reducing sugars in roots increased while that of starch decreased upon mycorrhization. In the functional symbiosis leaves showed higher levels of total soluble sugars, sucrose, reducing sugars, and starch (Nemec and Guy 1982; Dixon et al. 1988). Maize colonized by *Glomus* contained less starch than corresponding P_i-fertilized plants (Pacovsky 1989b).

In summary, in most cases of AM starch levels in leaves/whole plants were reduced while the amount of sugars contained in infected roots was higher in mycorrhizal systems than in non-mycorrhizal controls. The few existing data reported for ECM indicate the opposite. Here, mycorrhization coincides with decreased pools of soluble carbohydrates. If this possible difference between both types of mycorrhizas can be verified with other plant/fungus combinations it should reflect different ways of host/fungus interactions.

The AM data are interesting with respect to Björkman's (1949) carbohydrate theory for mycorrhizal development which assumes that "mycorrhizae

develop characteristically if the roots of the host plant contain a surplus of soluble carbohydrates". This theory is, however, based on observations with ECM. Also, in the case of increased carbohydrate pools, the causal sequence is a matter of debate (see Nylund 1988). Performing split-root experiments with beech and pine, Meyer (1962) found increased amounts of soluble carbohydrates in infected roots, but came to the opposite conclusion. He suggested infection first and then enhanced assimilate allocation by the host.

According to current knowledge, a higher sink strength should be caused by decreased root levels as found in the experiments with spruce and wheat (see also sect. 2.5.1). This discrepancy and the quite heterogeneous set of data could be due to different stages of mycorrhizal development investigated or the lack of knowledge on the compartmentation of reducing sugars (not sucrose) between the partners.

2.4.2 Photosynthesis / Leaf Metabolism

Gas exchange measurements under water stress showed higher stomatal conductance and net photosynthesis rate per unit leaf area in AM plants compared to non-mycorrhizal controls (Allen et al. 1981; Stahl and Smith 1984; Augé et al. 1986). This is obviously not directly related to drought conditions but to an enhanced demand for photoassimilates by ECM as well as AM (Allen et al. 1981; Paul and Kucey 1981; Ekwebelam and Reid 1983; Reid et al. 1983; Nylund and Unestam 1987; Brown and Bethlenfalvay 1988; Miller et al. 1989; Nylund and Wallander 1989; Clapperton and Reid 1992). This is in agreement with many observations that sink strength for photoassimilates can control the rate of photosynthesis (e.g. Herold 1980; Robbins and Pharr 1988). The interaction between fungus and plant is, however, dependent on the fungal partner. In ECMs formed between Douglas fir seedlings and *Rhizopogon vinicolor*, *Laccaria laccata*, or *Hebeloma crustuliniforme*, only *Rhizopogon* complied with this concept (Dosskey et al. 1991). The latter symbiosis did not affect plant dry mass but strongly increased the rate of net photosynthesis. In contrast, plants colonized with *Hebeloma* or *Laccaria* showed some suppression of growth but only a marginal enhancement of net photosynthesis. In *Pinus sylvestris* ECMs the fungal partners enhanced rates of photosynthesis, but *L. bicolor* was more effective than *H. crustuliniforme* at reducing host growth and enhancing photosynthesis (Nylund and Wallander 1989).

The rate of photosynthesis, on the other hand, affects the degree of mycorrhization. Citrus plants grown under long-day photoperiods had greater rates of basipetal transport of photoassimilates, which was paralleled by an improved mycorrhizal infection (Johnson et al. 1982b). Under reduced illumination additional drain of photoassimilates by the fungal partner can reduce the growth response of mycorrhizal plants compared to that of non-mycorrhizal controls (Tester et al. 1985).

2.5 Assimilate Allocation

Carbon partitioning in a plant is always regulated by the relative sink strength of competing organs. This must not always be the mycorrhizal root. Early flower bud formation can create a strong sink such that limited carbon availability decreases mycorrhiza formation, as shown for AM (Johnson et al. 1982a).

Movement of substances between component organisms is a primary feature of symbiosis and has its impact also on growth properties of the host plant. Plants colonized by, e.g. AM fungi have root systems which acquire a greater percentage of photoassimilates than non-mycorrhizal roots (Koch and Johnson 1984; Douds et al. 1988; Wang et al. 1989; in Clapperton and Reid 1992). Consequently, they exhibit higher root/shoot ratios (Clapperton and Reid 1990, 1992). By using carbohydrates for storage, for the building up of biomass, and for conversion into metabolic energy mycorrhizal fungi create strong assimilate sinks (e.g. Dosskey et al. 1990, 1991). This requires both specific structures and functions. As pointed out by Bracker and Littlefield (1973), morphological adaptations are of a dynamic nature and are subject to variation with type and duration of interaction. In ECM as well as in AM, intercellular transport in the apoplast constitutes an important part of the overall exchange of solutes which then have to cross the plasma membranes of both partners. This is also true for AM where only the host cell wall is penetrated while the host plasma membrane remains intact. A detailed review on this subject is given by Smith and Smith (1990). This infers that photoassimilates are unloaded from the phloem into the apoplast where they are open for use by both organisms. Possible losses from this "uncontrolled" space may be reduced by the endodermis and a suberized hypodermis (Shishkoff 1987; Smith et al. 1989). Controlled exchange of solutes, however, takes place across the opposed plasma membranes either via a cell wall interphase (ECM) or in direct membrane contact (AM). With regards to carbohydrate exchange this requires both availability of substrates for transport and the respective transport system.

2.5.1 Transfer of Carbohydrates

First labelling experiments with $^{14}CO_2$ in order to study carbon transfer were performed by Melin and Nilsson (1957). Generally, tracer studies indicate rapid translocation of ^{14}C-labelled assimilates to the roots of ectomycorrhizal plants (Cox et al. 1975), especially in young symbiotic interactions (Cairney et al. 1989).

The main transport form for photoassimilates in higher plants is sucrose (Ziegler 1975; Giaquinta 1983). Lewis and Harley (1965c) showed that labelled sucrose applied to the cut axis of excised beech mycorrhizas was translocated to the tip. Label accumulated particularly in the fungal sheath and was

incorporated into the fungal carbohydrates trehalose, mannitol, and glycogen. In seedlings of *Pinus sylvestris* infected with *Suillus variegatus* label from photosynthetic $^{14}CO_2$ fixation was detected in the cortex, Hartig net, and the hyphal mantle covering the fine roots (Bauer et al. 1991). Interestingly, nutrient solutions containing malt extract or glucose enhanced the growth of the fungus, although labelled photoassimilates were still accumulated in the mantle.

An attempt to assay the longitudinal distribution of soluble carbohydrates was made by Rieger et al. (1992). Lyophilized mycorrhizas (*Picea abies Amanita muscaria*) and fine roots of spruce (length < 2 mm) were dissected into about 0.5 mm thick slices which represented four zones of different physiological functions. A longitudinal distinction of pools of sucrose, glucose, and fructose showed a specific response of sucrose. This saccharide showed the least amounts in the middle parts of a mycorrhiza, i.e. the area of most intense symbiotic interaction (Fig. 3). Fine roots without fungal infection, in contrast, did not show longitudinal variations in sugar content. Reasons for this finding could be both enhanced sucrose consumption, leading to an increased sink strength, or "tissue dilution" by the fungal partner. The latter can be considerable. In the area of interaction the fungal partner can add up to 50% of the total dry weight of a mycorrhiza. As fungal hyphae obviously do not take up sucrose (see above and below), all the sucrose should be confined to the host tissue, which finally should contain an amount of sucrose comparable to that of uninfected roots. There was, however, also a jump in sucrose content between carrier and fine roots of uninfected material which could be sufficient to create the necessary sink strength for photoassimilate partitioning.

Mycorrhization can also be influenced by radial carbohydrate gradients in roots as shown by the preferred location of arbuscules at the inner cortex of some AM, where carbohydrate concentrations were highest (see Koide and Schreiner 1992).

An increased sink activity for photoassimilates is also created in AM roots (Bevege et al. 1975; Lösel and Cooper 1979), and the degree of infection of a particular root is obviously more dependent on its carbohydrate supply than on the number of infection fronts (Buwalda et al. 1984). In this symbiosis different fungal products are formed. Lipids have been reported to be particularly important in mature arbuscules, vesicles and hyphae, and glycogen granules are commonly associated with lipid structures (for a review, see Smith and Gianinazzi-Pearson 1988).

Sucrose unloading to root tissue has also been shown for ECM-forming conifers (Komor 1983), but there is controversy about how the fungal partner can make use of it. As far as the culture of mycorrhizal fungi has been examined, growth on sucrose, in contrast to fructose and glucose, was very poor (see above). This gave rise to the conclusion that the respective fungal cells either lacked a transport system for sucrose or did not contain sucrose clearing enzymes such as invertase or sucrose synthase. Growth of, e.g. the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* on

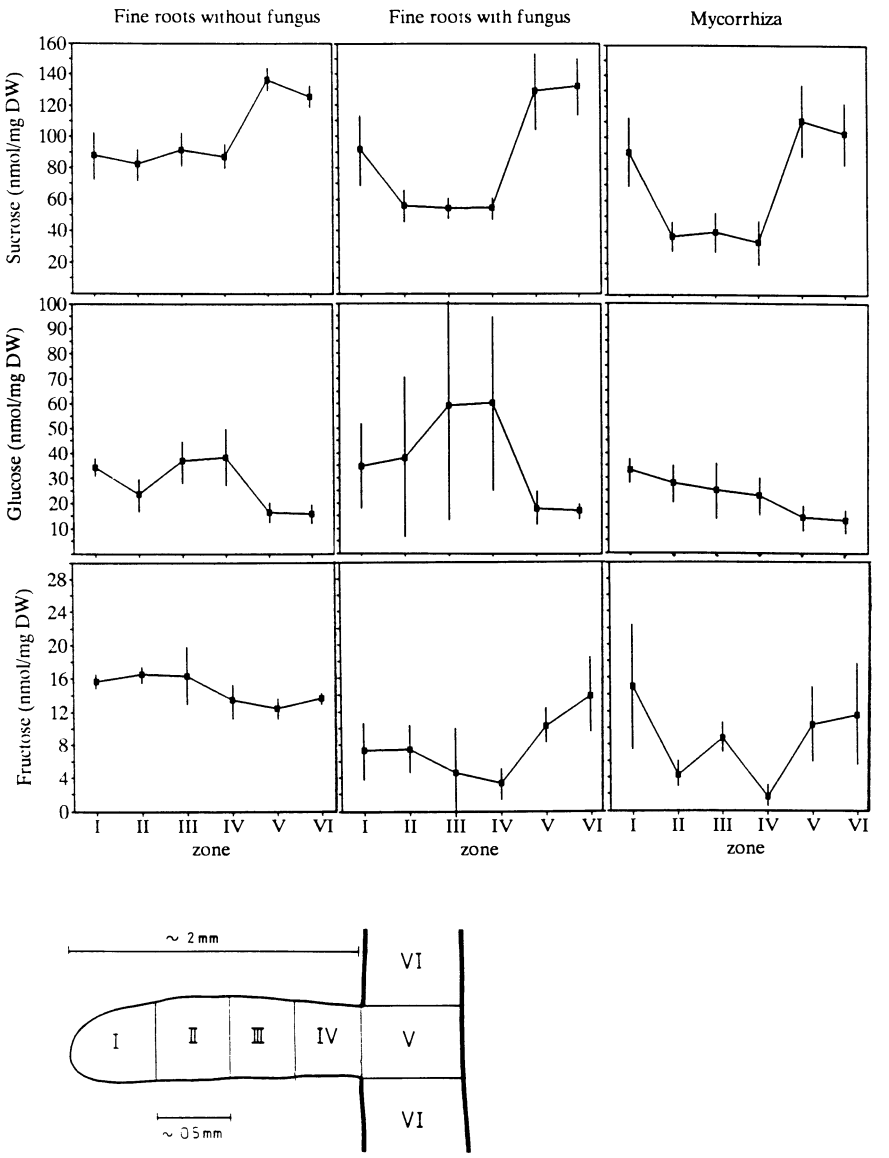

Fig. 3. Content of soluble carbohydrates in six distinct zones of spruce roots. Roman numbers (I to VI) refer to tissue slices cut from fine roots as shown in the schematic drawing. Fine roots "without fungus" refer to non-inoculated spruce seedlings, while "fine roots with fungus" are taken from inoculated Petri dishes without showing hyphal mantles. Sucrose levels of the latter are intermediate to those of uninfected roots and of mycorrhizas. They could thus represent an early stage of infection with no externally visible signs. Means ± S.D. (n = 3)

sucrose was only possible after addition of invertase (Salzer and Hager 1991). Root cell walls contain acid invertase (for suspension culture cells and root tissue of spruce, see Salzer and Hager 1991). It is therefore very probable that sucrose delivered by the host is hydrolyzed in the root apoplast by host enzymes. The products of hydrolysis, glucose and fructose, are obviously not taken up at the same rates. The ECM-forming basidiomycetes *Amanita muscaria* and *Hebeloma crustuliniforme* grew better on glucose than on fructose (Salzer and Hager 1991). A direct assay of sugar uptake employing [^{14}C]-labelled substrates and protoplasts from *Amanita muscaria* confirmed this report. K_M values for the uptake of glucose and fructose were 1.25 and 11.3 mM, respectively. In addition, glucose uptake was only marginally affected by fructose, while glucose was highly competitive with regards to fructose (Chen and Hampp 1993). As glucose is the substrate giving rise to the formation of fungus-specific trehalose, the preferred uptake of this hexose could be specific for basidiomycetes which appear to accumulate trehalose instead of mannitol (see above and Fig. 4). If this assumption is true, mannitol-accumulating ascomycetes should possess a transport system with a higher affinity for fructose. Transport studies similar to those reported by Chen and Hampp (1993) but with *Cenococcum geophilum* did not support this assumption as this ascomycete exhibited kinetics of sugar transport comparable to those of *Amanita muscaria* (Hampp et al. 1995).

Uptake of hexoses by the fungus must not necessarily be energy-dependent as it could be along a concentration gradient due to the conversion into fungal carbohydrates. There is, however, evidence for active transport. Uptake of glucose and fructose by *Amanita* protoplasts was highly dependent on the intracellular ATP/ADP ratio. Depletion of ATP by inhibitors of respiratory ATP formation significantly decreased the rate of uptake (Chen and Hampp 1993). Membrane-bound ATPase activities which could be involved in sugar transport (for higher plants, see e.g. Hager et al. 1986; Serrano 1988) have been detected in the Hartig net area of ECM (*Pinus sylvestris/Laccaria laccata*; Lei and Dexheimer 1988) and show high rates on the arbuscules in AM (Marx et al. 1982; Gianinazzi-Pearson and Gianinazzi 1989). Schiebel (1988) gave physiological proof for the existence of H^+-translocating ATPases in cultures of *A. muscaria* and *H. crustuliniforme*. Their immunological detection was performed by Saile (1990).

There are other suggestions about the mechanism of carbohydrate transfer at the arbuscular interface of AM. According to Schwab et al. (1991), mycorrhizal partners could exchange P_i and carbohydrates via a translocator comparable to the P_i translocator at the inner chloroplast envelope (Flügge and Heldt 1991). Regardless how attractive such a mechanism would be, there is not much evidence for such an exchange (for criticism, see Smith and Gianinazzi-Pearson 1988; Harley 1991).

Most recently, cDNA clones coding for hexose transporters have been isolated. One was identified from a library prepared from *Medicago trunculata* roots which were colonized by *Glomus versiforme* (Harrison 1996). The clone

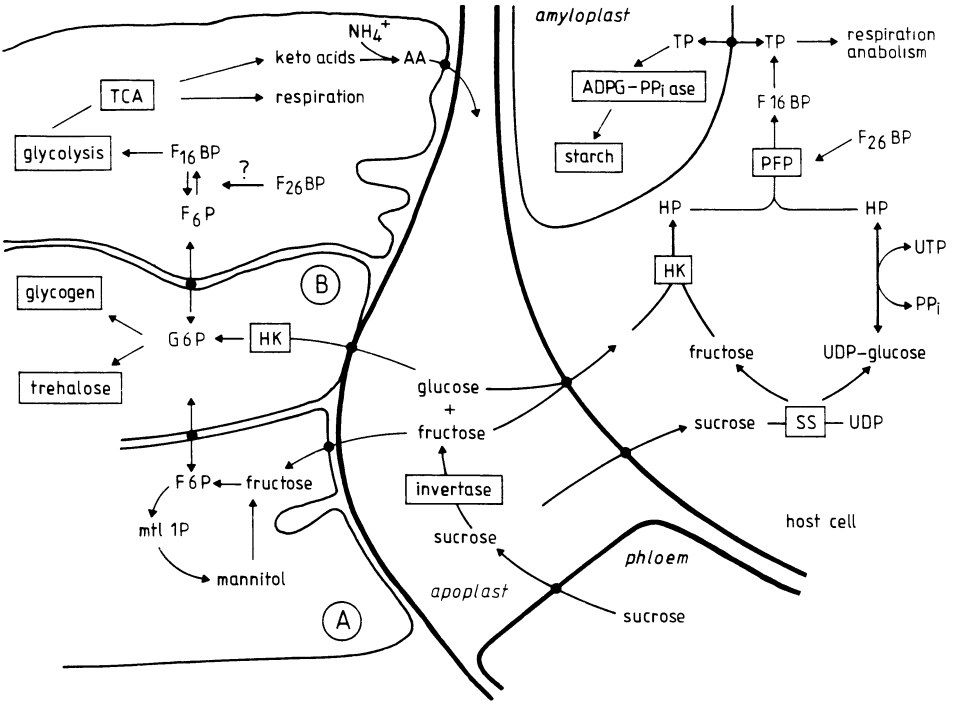

Fig. 4. Possible ways of sucrose metabolism in ectomycorrhizal roots. Fungal cells (left) are opposed to a host cell (right). The mannitol cycle is fully operative in ascomycetes (A) only while the formation of trehalose dominates in basidiomycetes (B). The function of fructose 2,6-bisphosphate (F26BP) has not been verified, yet. AA, amino acids; ADPG-PP_iase, ADP-glucose pyrophosphorylase; HK, hexokinase; mtl 1P, mannitol 1-phosphate; PFP, PP_i-dependent fructose 6-phosphate phosphotransferase; PP_i, pyrophosphate; SS, sucrose synthase; TCA, Krebs cycle; TP, triose phosphates

represented a host gene, and expression studies in yeast indicated a transport specificity for hexoses, not for sucrose. The transporter was expressed in leaves, stems, and (highest) in root tips. In the latter, transcript levels increased up to four-fold upon colonization by *Glomus versiforme.*

Similar studies with an ectomycorrhizal system (spruce/fly agaric) indicate unaltered expression of a host root hexose transporter but increased expression (4 times) of the fungal counterpart upon establishment of symbiotic interaction in a hexose dependent manner (Nehls et al. 1998).

2.5.2 Effects of Mineral Nutrition

2.5.2.1 P_i Supply

Obviously, carbon allocation is dependent on P_i supply. Increased P_i availability has been shown to limit carbon export to the root (see Koide and Schreiner

1992). With roots of subterranean clover increasing P_i supply decreased both the percentage of root length converted into mycorrhizas and the concentration of soluble carbohydrates (Same et al. 1983). Shading, defoliation and low root temperatures had the same effect (Same et al. 1983) and trehalose was one of the carbohydrates being most responsive (Schubert et al. 1992). Thomson et al. (1986) showed that P_i nutrition causing reduced levels of root soluble carbohydrates also led to decreased growth of fungi outside the root in the AMs formed between *Trifolium subterraneum* and *Gigaspora caulospora/ Glomus fasciculatum*.

On the other hand, limiting P_i levels have been shown to increase the degree of carbohydrate exudation from roots (see Koide and Schreiner 1992). This could render roots more attractive for fungal infection and thus increase mycorrhization. There are, however, also reports that, at comparable P_i availability, AM plants exhibit higher concentrations of extractable carbohydrates (Thomson et al. 1986, 1990). Thus, AM fungi may increase the carbohydrate pools of corresponding root tissues which is not necessarily the case with ECM (see sect. 2.5 and 2.5.1). Data from Nylund's laboratory (Wallander and Nylund 1991; Wallander 1992, and pers. comm.) suggest a threshold effect in the mycorrhizal response to P_i. Sugar supply towards the fungus increases only below the threshold (P_i starvation).

It should be mentioned that increased P_i nutrition should not decrease but rather increase photoassimilate partitioning towards sink organs such as mycorrhizas. Possible reasons for decreased assimilate supply in ECM are discussed in section 2.5.3.

2.5.2.2 N Supply

The interaction of nitrogen supply, carbohydrate availability, and mycorrhiza formation has been the subject of several studies (see Wallander and Nylund 1991). There is biochemical evidence that plants with an ample nitrogen supply have less carbohydrates available for distribution which can result in increased shoot/root ratios (Radin et al. 1978; Stroo et al. 1988; see also sect. 2.5.3) and decreased degrees of mycorrhization.

Pot experiments with 3-year-old spruce seedlings on soils with increasing N supply indicated a shift from starch formation or sucrose export (needles) toward amino acid production. In parallel, fungal markers such as ergosterol, trehalose or mannitol were decreased in root samples, indicative of a reduced allocation of photoassimilates toward the fungal partner (Wallenda et al. 1996). Depletion of roots of soluble carbohydrates by increased N supply could thus make them less attractive for fungal infection. This hypothesis, however, possibly can not be generalized, and it is also reasonable to assume that the decrease in fungal growth under high nitrogen supply is due to an increased fungal production of amino acids which are relocated to the host tissue (allocated carbon skeletons are thus not available for fungal growth). Amino acid formation depends on the supply of carbon skeletons in form of ketoacids. These are derived from intermediates of glycolysis (phosphoenol

pyruvate) by carboxylation ("anaplerotic pathway"). A comparative analysis of activities of carboxylating enzymes in non-mycorrhizal and mycorrhizal roots of spruce showed a clear shift from root tissue toward the fungal partner upon mycorrhiza formation (Wingler et al. 1996). Thus, under increased N supply the fungus could indeed suffer from high rates of amino acid production and export. Suggestions and working hypotheses in order to help in the understanding of effects of N supply on ECM formation are given by Wallander (1995).

Obviously, the effect of increased N nutrition not only depends on the availability of N, but also on other parameters such as water supply and photosynthetic capacity. If the latter is high enough to support the need of additional carbon skeletons for nitrogen assimilation (low stomatal resistance, high photon flux density), then carbon partitioning within the plant, and thus mycorrhiza formation, should not be affected (see also elevated supply of CO_2; 2.7.2).

2.5.3 Regulation of Assimilate Allocation

2.5.3.1 Source Tissue

Regulation of mycorrhiza under the influence of carbohydrates can be with regards to both formation and function. The involvement of photoassimilates in the initiation and maintenance of a mycorrhiza (Björkman's carbohydrate theory: root sugar concentration must reach a certain threshold value before a mycorrhization can take place) has been thoroughly reviewed by Nylund (1988) and has been addressed above. This complies with evidence that plants with an ample nitrogen supply have less carbohydrates available for distribution and are thus less attractive for fungal infection.

In an established mycorrhiza, regulation of fungal growth is important for the mutual benefit of the symbiosis. If assimilate extraction from the plant by the fungus becomes too extensive, a decrease in plant performance and fitness could result. For AM there is evidence that the host plant actively regulates infections in order to optimize its fitness. Limited light, e.g., which results in a decrease in photoassimilate production, often reduces the degree of mycorrhizal infection (see Koide and Schreiner 1992). Mechanisms involved in the host-dependent regulation of this symbiosis may be by chemical stimuli, chemical or structural mechanisms to limit the degree of infection, or by the delivery of carbohydrates (Koide and Schreiner 1992).

With regard to the supply of carbohydrates this could include regulation at the level of sucrose synthesis in source organs, phloem loading of photoassimilates [i.e., partitioning between source and sink (root) organs], sucrose hydrolysis and assimilate transfer in the area of solute exchange.

Regulation in source organs starts at the level of assimilate partitioning between starch and sucrose, and the rate of starch synthesis in a source leaf is

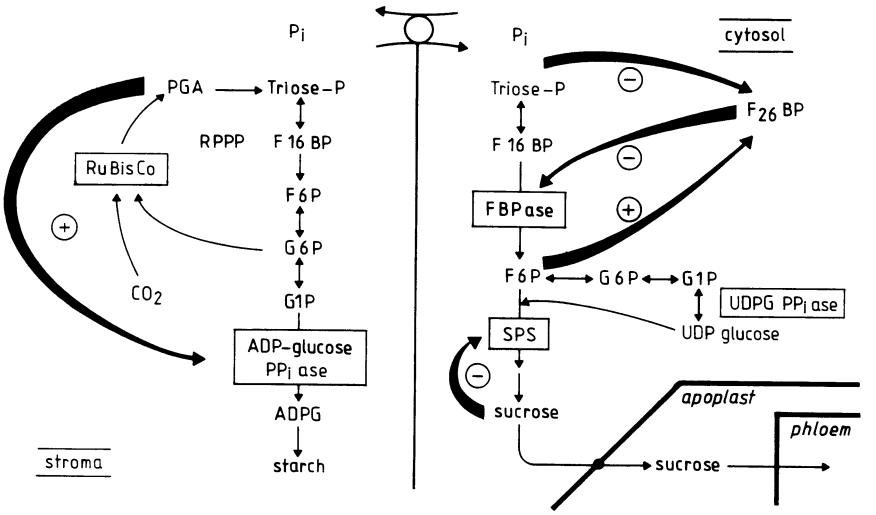

Fig. 5. Carbon allocation in a mesophyll cell. Regulatory interactions are marked as +/−. FBPase, fructose 1,6-bisphosphatase; PP_iase, pyrophosphorylase; RPPP, reductive pentose phosphate pathway; SPS, sucrose phosphate synthase; UDPG, UDP-glucose

controlled by the rates of sucrose formation and export. This control is mediated by a complex coordination of metabolic processes in two subcellular compartments of the leaf mesophyll, the chloroplast and the cytosol (Stitt 1990). F26BP, an important cytosolic regulator of glycolysis and present only in micromolar concentrations (Steingraber et al. 1988), affects cytosolic sucrose synthesis by inhibiting the fructose 1,6-bisphosphatase reaction (sucrose synthesis) and activating a PP_i-dependent phosphofructokinase, thus enhancing glycolysis (Fig. 5). Metabolic intermediates of glycolysis such as triose phosphates or fructose 6-phosphate can affect the cytosolic levels of F26BP.

If, for example, rates of sucrose synthesis are higher than the vacuolar capacity for storage or than the rates of export, sucrose would accumulate. This is avoided by decreasing the activity of sucrose phosphate synthase (Kerr and Huber 1987). A consequent increase in the F6P pool will then stimulate the synthesis of F26BP which, in turn, will inhibit FBPase activity and thus sucrose formation (Stitt 1990). In the presence of increased cytosolic TP levels the regulation can be in the opposite direction. TP can inhibit F26BP formation and hence relieve the inhibition of FBPase, an event which finally will support sucrose formation.

The availabilty of carbon skeletons for sucrose synthesis and thus for transport to sinks such as mycorrrhizal roots depends on nutrition. Owing to the dependency of assimilate (TP) export from the chloroplast on the cytosolic P_i pool (P_i translocator; Flügge and Heldt 1991), the supply of P_i regulates the allocation of newly fixed carbon between starch and sucrose. Depletion of P_i will thus favor the formation of chloroplast starch. Consequently, an increased

supply of P_i by symbiotic interaction at the root level should also enhance the carbon supply to the root, owing to improved sucrose formation and export. This conclusion from the regulatory point of view is, however, not consistent with investigations concerning P_i availability and degree of mycorrhization, where increasing P_i nutrition decreased root levels of soluble carbohydrates and the formation of mycorrhizas (see sect. 2.5.2). An explanation for this descrepancy could be that under conditions where P_i is not limiting sink activities such as buds, flowers, etc. are created which are stronger than those of roots, thereby diverting sucrose away from roots.

In the presence of surplus nitrogen (NH_4^+) the supply of sucrose to the roots can also be reduced, but for different reasons. In experiments with algae and cell suspension cultures of higher plants it has been shown that the addition of inorganic nitrogen leads to an increased synthesis of amino acids which, eventually, can lead to decreased rates of sucrose and starch synthesis (Kanazawa et al. 1970, 1972; Larsen et al. 1981; Turpin et al. 1990). Provision of inorganic nitrogen to barley leaf segments and spinach leaf discs led to an increased production of amino acids and an inhibition of sucrose synthesis (Champigny et al. 1992). The increased carbon flux to amino acids was associated with a decrease in phosphoenol pyruvate which was taken as evidence that phosphoenol pyruvate carboxylase and/or pyruvate kinase were activated. In addition, F26BP (see Fig. 5) increased by up to 100% and sucrose phosphate synthase activity declined (spinach). Similar changes were found with cotyledons of germinating *Ricinus* seedlings (Geigenberger and Stitt 1991).

Nutrition of 5-month-old spruce seedlings with excess nitrogen (three times the amount of a standard Ingestad medium; Ingestad and Kähr 1985) resulted in significantly reduced starch levels of needles. In parallel, F26BP was significantly increased (Einig et al. 1993). The response of spruce needles should thus represent a comparable diversion of photoassimilates into anaplerotic reactions as reported by Champigny et al. (1992).

Such a response upon increased N nutrition would certainly influence mycorrhization. Obviously, under these conditions metabolism of spruce needles also switches to an increased anaplerotic activity to maintain the supply of carbon skeletons for amino acid synthesis. As the capacity for sucrose formation and export is most probably diminished, carbon allocation toward sink organs such as roots will be affected. This would explain the decrease in mycorrhization.

2.5.3.2 Fungus

In order to gain photoassimilates, the fungal partner has to establish some sink quality. This can be achieved by conversion of host derived sugars into fungus-specific compounds (trehalose, mannitol, glycogen; see above), or by increased metabolic turnover. Most recently, Schaeffer et al. (1996) could show that the regulation of fungal glycolysis is completely different from that of host cells. In

Amanita, like in yeast or animal cells and in contrast to plant cells, the glycolytic key enzyme, ATP-dependent phosphofructokinase, is highly activated by F26BP (k_a about 30 nM). As mycorrhizal roots have increased contents of F26BP (Hampp et al. 1995), it could be assumed that fungal glycolysis is upregulated in spruce/fly agaric mycorrhizas. This will cause increased rates of glucose consumption and thus improve the sink quality of a mycorrhiza. In yeast cells, glucose activates glycolysis via an increase of the concentration of a second messenger, cyclic AMP, which in turn causes an increase of the F26BP concentration (Hers and Van Schaftingen 1982). In culture experiments with *Amanita muscaria* we could show that upon addition of glucose the amount of cyclic AMP, and somewhat later, also that of F26BP, increases (Hoffmann et al. 1997). Thus we hypothesize that host derived glucose triggers fungal metabolism and thus could stimulate carbon flow toward the fungal partner in an ECM.

2.6 Seasonal Changes

In addition to their function as mediators for assimilate transport and storage, soluble carbohydrates are also involved in the protection against desiccation by increasing the osmotic potential (Guy 1990). An attempt to analyze seasonal changes in the pool sizes of soluble carbohydrates in spruce mycorrhiza was made by Niederer et al. (1992). Sucrose, raffinose, trehalose, and mannitol were high in winter and low in summer. Largest seasonal changes were observed with raffinose. Exposure of excised mycorrhiza to low temperatures or drought significantly increased the level of trehalose, which has been shown to be especially effective in protecting against desiccation stress (Crowe et al. 1984).

2.7 Effects of Environmental Stress

There abundant evidence that physiological stressors such as, e.g. low doses of air pollutants, can significantly change source/sink relationships in a complex way, most commonly increasing the shoot/root ratio (for overviews, see Koziol et al. 1988; Smith 1990; Tingey and Andersen 1991; Hampp 1992). A decreased supply of photoassimilates to the root system should most certainly affect mycorrhizal development, and there is biochemical evidence for altered phloem loading of sucrose in spruce trees showing symptoms of decline (Einig and Hampp 1990).

2.7.1 Pollutants

Reports on ozone effects on mycorrhiza are conflicting. For example, mycorrhizal infection of northern red oak seedlings increased following ozone expo-

sure (Reich and Amundson 1985; Reich et al. 1985), whereas no effect on ectomycorrhiza of loblolly pine was detected (Mahoney et al. 1985). Ho and Trappe (1984) reported a decreased mycorrhizal frequency after treatment of *Festuca arundinacea* with ozone. Juvenile Douglas fir inoculated with *Rhizopogon vinicolor* and *Lactarius rufus* when treated with 200 μg ozone/m^3 for 28 days exhibited a decreased allocation of ^{14}C to the root system after $^{14}CO_2$ fixation (Gorissen et al. 1991). Mycorrhizal frequency, however, tended to increase.

Tomato seedlings inoculated with *Glomus fasciculatum* and exposed to ozone for 3 h twice a week exhibited a reduction in mycorrhizal infection by 46 and 63% at 150 and 300 ppb (300/600 μg/m^3) O_3 (McCool and Menge 1983). This was connected to decreased root contents of reducing sugars and to a significant loss in total dry weight in comparison to non-mycorrhizal plants. Under such conditions non-inoculated plants obviously benefit from not having to share carbohydrates under conditions of restricted allocation to the roots.

Exposure of loblolly pine seedlings to up to 150 ppb (300 μg/m^3) O_3 (5 h/day; 5 days/week; for 6 or 12 weeks) resulted in a linear dose-response relationship with regard to mycorrhiza formation (Meier et al. 1990). Both exposure to ozone and soil water deficit decreased the amount of available carbohydrates, but only ozone caused a suppression of ectomycorrhizas.

In summary, there is good evidence that ozone at concentrations which are easily reached on sunny summer days (100 to 200 μg/m^3) decreases carbon translocation to the root system, thereby decreasing the probability for mycorrhiza formation.

Exposure of *Phleum pratense*, colonized by AM and exposed to SO_2 (0.04–0.065 μl/l) for 6 weeks caused a significantly reduced proportion of ^{14}C-labelled soluble carbohydrates in the roots upon $^{14}CO_2$ assimilation (Clapperton and Reid 1992). This agrees well with the observation that SO_2 fumigation inhibits both phloem loading and rates of assimilate transport out of leaves (Gould et al. 1988). In parallel, the root length colonized by AM fungi and the length of root infected with arbuscules were reduced.

Interaction of air pollutants and acid precipitation should increase the complexity of responses. A combination of both applied on loblolly pine seedlings had no statistically significant effects on carbon allocation (determined via fixation of $^{14}CO_2$), but root starch and the degree of mycorrhizal infection varied significantly with ozone levels (Adams and O'Neill 1991).

Soil acidification due to acid rain is a factor potentially affecting the degree of mycorrhization. Mycorrhizal colonization of roots of *Pinus strobus* with *Pisolithus tinctorius* was suppressed under acid precipitation while plant growth was not affected (Stroo and Alexander 1985). Stroo et al. (1988) found a linear relationship between the decreasing number of mycorrhizal short roots per lateral *Pinus strobus* root and decreasing rain pH. Erland et al.

(1991) determined the loss of activity from roots of *Pinus sylvestris* and *P. contorta* at pH 3.8 and 5.2 after ^{14}C labelling of photoassimilates. They found that loss to the medium was more rapid from mycorrhizal roots and that transfer of label to mycelia growing at pH 3.8 was faster than at pH 5.2. Thus, decreasing rhizosphere pH could increase the rate of consumption (respiration?) by the fungus of allocated carbon which could decrease the availability of photoassimilates for anabolic processes such as the formation of new symbiotic interactions.

2.7.2 CO_2

The response of plants, and especially ECM-forming trees, toward elevated CO_2 depends on nutrient availability and the degree of utilization of photoassimilates. There are at least short-term responses of metabolism and carbon allocation (Eamus and Jarvis 1989; Mousseau and Saugier 1992) which apply mainly to plants with limited sink capacities. These plants do not take advantage of an increased supply of CO_2 because there is a feedback regulation (decreased gene expression) of carbon fixation by accumulating sugars (Stitt 1991; Koch 1996). Mycorrhizal plants which are depleted of photoassimilates by their fungal partner should therefore perform better under conditions of increased CO_2. Indeed, seedlings of *Pinus echinata* exhibited a higher degree of root colonization by mycorrhizal fungi when grown under elevated CO_2 (Norby et al. 1987). Similarly, pine seedlings inoculated with the ECM-forming fungus *Pisolithus tinctorius* exhibited a much faster fungal growth under elevated CO_2 ($600\,\mu mol\,mol^{-1}$), while no effect on shoot biomass was found (Ineichen et al. 1995).

Better mycorrhization improved P_i and K uptake and thus supported growth under higher CO_2 (Norby et al. 1986; Lewis and Strain 1996).

2.7.3 Drought

Mycorrhization may improve the plant water status. Under comparable water limitation mycorrhizal plants had higher rates of transpiration and a higher leaf turgor than non-inoculated ones (see Andersen and Rygiewicz 1991). Under conditions that support mycorrhiza formation ECM or AM plants should, however, also exhibit an improved nutritional status and a different allocation pattern of photoassimilates (see above). They are thus not directly comparable to non-inoculated plants and direct effects of mycorrhization on plant water relations are difficult to assess. As far as the content of soluble sugars is increased in roots or leaves of mycorrhizal plants (see sect. 2.4), this could support osmotic adjustment in order to compensate for a decreased soil water potential.

3 Energy Metabolism

Mycorrhizal roots have been shown to have higher respiratory rates than uninfected ones (Snellgrove et al. 1982; Harley and Smith 1983; Baas et al. 1989), although this can be highly variable (Nylund and Wallander 1989). At least part of this increase should be due to the fungal hyphae.

Calculations of the quantity of carbon necessary for the maintenance of ectomycorrhizal fungi assume that about 10% of the net production of assimilates is consumed for this purpose (Harley 1991). Including transport of assimilates between partners, carbon needs have been suggested to be up to 30% and more (see Kozlowski 1992). It is, however, quite possible that infected root cells themselves respire at enhanced rates because of increased needs of energy for solute translocation. There is at least electron microscopic evidence for this assumption. AM infected root cells have been shown to possess a higher cytoplasmic volume, more mitochondria, more soluble protein, and increased activities of metabolic pathways (Cox and Sanders 1974; Smith et al. 1985).

Glucose breakdown via glycolysis and the oxidative pentose phosphate pathway (OPPP) have been reported for spruce/*Hebeloma* mycorrhizas and evidence exists that the contribution of the OPPP to respiration is higher in the fungal partner (Bilger et al. 1989). OPPP activities in extramatrical hyphae were lower compared to those found in host tissue colonized by fungal hyphae. From this the authors suggested that this pathway is stimulated in the symbiotic fungus. According to Martin and Hilbert (1991), this should even lead to a reduced requirement of energy production by host cells ("reduced rate of basal respiration") as the fungal cells take over energy-consuming tasks such as uptake, reduction and transport of solutes.

The exchange of nutrients taking place between plant and fungus depends on the maintenance of concentration gradients across membranes (see above for carbohydrates) and should thus require metabolic energy. It is therefore reasonable to assume that pool sizes of adenylates or even the energy status of root tissue sections involved in symbiotic activity should deviate from that of non-mycorrhizal fine roots. One of the major changes during the formation of vesicular-arbuscular mycorrhizas is obviously an increase in cytoplasmic volume of the interacting cells (Cox and Tinker 1976; Dexheimer et al. 1979) which could be taken as evidence for an increased metabolic activity. Wieser et al. (1986) compared pool sizes of adenylates in roots of onion grown in the presence or absence of *Glomus fasciculatum*. Infected roots showed a total pool of adenylates (ATP + ADP + AMP) which was consistently about three times higher than in non-infected ones. Interestingly, this effect was not connected to the number of functional arbuscules as the increase in pool sizes was already established at the onset of infection and remained constant during the intensification of the symbiotic interaction. The authors discussed this early event as being more likely a kind of metabolic stimulation of the host tissue

than an indication of increased metabolic capacity for energy requiring metabolite exchange. Calculations of the adenylate energy charge ([ATP + $^1/_2$ADP]:[ATP + ADP + AMP]) given in their Table 1, however, revealed no significant difference between both systems. In contrast, the ratio of ATP/ADP (calculated from their data), which can also be taken as a measure of the energy status, was slightly higher in mycorrhizal roots (approx. 15%).

Trials to assess pool sizes of adenylates along fine roots at different degrees of mycorrhizal interaction were performed with ectomycorrhizas established between spruce and fly agaric (Namysl et al. 1991). Using frozen-dried material and dissectioning techniques (see Rieger et al. 1992) four zones from tip to base of controls and infected fine roots were distinguished and compared. In this case, the ratio of ATP/ADP of zones involved in mutual interaction between both partners (Hartig net region) was higher compared to non-infected samples. This is in accordance with the assumption of an increased energy need in this interactive region and the higher rates of respiration found in mycorrhizal roots in general.

There is also a possible relationship between the viability of ectomycorrhizal fungal inoculum and its energy status (Lapeyrie and Bruchet 1985). A blended inoculum prepared from *Pisolithus tinctorius* grown in liquid culture had a maximum activity at an age when its ATP content was highest, too. This observation should, however, be treated with caution as the authors only assayed ATP. The ATP pool alone can vary considerably, and an interpretation of its physiological/biochemical significance can only be made in the context with, at least, ADP.

4 Conclusions

Supply of carbohydrates by the host is the basis for the development of a functional mycorrhiza, and allocation of host photoassimilates to the interactive root areas is triggered in several ways. Most important are obviously establishment and maintenance of gradients between both partners, and between root and shoot. These are achieved by either conversion of, e.g. host sucrose into fungus-specific compounds such as trehalose or mannitol, or consumption by fungal growth and respiration. The latter appears important with respect to energy needs for transport processes across the plasma membranes of both partners and is possibly reflected by increased ATP/ADP ratios in interactive tissue regions. Carbohydrate needs of mycorrhizal roots obviously decrease sugar availability for the host as shown by reduced starch levels in the latter. The impact on host pools of soluble carbohydrates is, however, more variable and possibly different for both AM and ECM.

There is now sufficient evidence that the needs for photoassimilates in a developing or functional mycorrhiza induce increased rates of photosynthesis. Such a response could be achieved merely by a steepened sucrose gradient

between shoot and root, affecting regulation of carbon partitioning between sucrose and starch and related mesophyll cell metabolite pools (possible regulatory events are discussed). The participation of other signals (hormones, etc.), however, can not be ruled out. The impact of nutrient supply (N, P_i) on carbon allocation and, subsequently, on mycorrhization could act via the same biochemical mechanisms, and examples for the possible effect of increased levels of N and P_i on carbon partitioning are given.

One of the major obstacles in investigating metabolic interactions in mycorrhiza is dimension. Owing to the small size, tissue-related metabolic distinction in, e.g. the Hartig net region is only possible via histochemical approaches, with quantitative histochemistry being most promising. Investigations into the translocation of metabolites of mutual interest (carbohydrates, amino acids) across the plasma membranes of both symbiotic partners are largely missing. The possibility to obtain viable protoplasts from mycorrhizal fungi as well as the cloning of genes of relevant metabolite transporters from both root and fungal cells, and their regulation by mycorrhiza formation, should stimulate further research in this field.

Acknowledgments. As far as our own results are presented, we gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft, the Bundesminister für Forschung und Technologie (EUROSILVA), and the Landesforschungsförderungsprogramm Baden-Württemberg. We are indebted to M. Guttenberger, A. Hager, E. Magel, U. Nehls, J. E. Nylund, T. Wallenda, and A. Wingler for critical reading and help in the preparation of this manuscript.

References

Abuzinadah RA, Read DJ (1986) The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. I. Utilisation of peptides and proteins by ectomycorrhizal fungi. New Phytol 103:481–493

Adams MB, O'Neill EG (1991) Effects of ozone and acidic deposition on carbon allocation and mycorrhizal colonisation of *Pinus taeda* L. seedlings. For Sci 37:5–16

Adams RP, Kendall E, Kartha KK (1990) Comparison of free sugars in growing and desiccated plants of *Selaginella lepidophylla*. Biochem Syst Ecol 18:107–110

Allen MF, Smith WK, Moore TS Jr, Christensen M (1981) Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis*. New Phytol 88:683–693

Andersen CP, Rygiewicz PT (1991) Stress interactions and mycorrhizal plant response: understanding carbon allocation priorities. Environ Pollut 73:217–244

Augé RM, Schekel KA, Wample RL (1986) Osmotic adjustment in leaves of VA mycorrhizal and non-mycorrhizal rose plants in response to drought stress. Plant Physiol 82:765–770

Avigad G (1982) Sucrose and other disaccharides. In: Loewus FA, Tanner W (eds) Plant carbohydrates. I. Intracellular carbohydrates. Springer, Berlin Heidelberg New York, pp 217–347

Azcón R, Ocampo JA (1981) Factors affecting the vesicular-arbuscular mycorrhizal infection and mycorrhizal dependency of 13 wheat *Triticum vulgare* cultivars. New Phytol 87:677–686

Azcón R, Ocampo JA (1984) Effect of root exudation on vesicular-arbuscular mycorrhizal infection at early stages of plant growth. Plant Soil 82:133–138

Baas R, Van Der Werf A, Lambers H (1989) Root respiration and growth in *Plantago major* as affected by vesicular-arbuscular mycorrhizal infection. Plant Physiol 91:227–232

Bauer T, Blechschmidt-Schneider S, Eschrich W (1991) Regulation of photoassimilate allocation in *Pinus sylvestris* seedlings by the nutritional status of the mycorrhizal fungus *Suillus variegatus*. Trees 5:36–43

Bevege DI, Bowen GD, Skinner MF (1975) Comparative carbohydrate physiology of ecto- and endomycorrhizas. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London, pp 149–174

Bilger I, Guillot V, Martin F, Le Tacon F (1989) Assessment of the contributions of glycolysis and the pentose phosphate pathway to glucose respiration in ectomycorrhizas and non-mycorrhizal roots of spruce (*Picea abies* L. Karsten). Ann Sci For 46:794–797

Björkman E (1949) The ecological significance of the ectotrophic mycorrhizal association in forest trees. Sven Bot Tidskr 43:223–262

Borges RG, Chaney WR (1989) Root temperature affects mycorrhizal efficacy in *Fraxinus pennsylvanica* marsh. New Phytol 112:411–418

Bracker CE, Littlefield LJ (1973) Structural concepts of host-pathogen interfaces. In: Byrde RJW, Cutting CV (eds) Fungal pathogenicity and the plant's response. Academic Press, London, pp 159–317

Brown MS, Bethlenfalvay GJ (1986) The *Glycine-Glomus-Rhizobium* symbiosis. III. Endophyte effects on leaf carbon, nitrogen and phosphorus nutrition. J Plant Nutr 9:1199–1212

Brown MS, Bethlenfalvay GJ (1988) The *Glycine-Glomus-Rhizobium* symbiosis. VII. Photosynthetic nutrient-use efficiency in nodulated mycorrhizal soybeans. Plant Physiol 86:1292–1297

Buwalda JG, Stribley DP, Tinker PB (1984) The development of endomycorrhizal root systems. V. The detailed pattern of development of infection and the control of infection level by host in young leek plants. New Phytol 96:411–427

Cairney JW, Ashford AE, Allaway WG (1989) Distribution of photosynthetically fixed carbon within root systems of *Eucalyptus pilularis* plants ectomycorrhizal with *Pisolithus tinctorius*. New Phytol 112:495–500

Champigny ML, Brauer M, Bismuth E, Cao Thi Manh, Siegl G, Le Van Quy, Stitt M (1992) The short-term effect of NO_3^- and NH_3 assimilation on sucrose synthesis in leaves. J Plant Physiol 139:361–368

Chen X-Y, Hampp R (1993) Sugar uptake by protoplasts of the ectomycorrihzal fungus *Amanita muscaria*. New Phytol 125:601–608

Clapperton MJ, Reid DM (1990) Effects of sulphur dioxide fumigation on *Phleum pratense* and vesicular-arbuscular mycorrhizal fungi. New Phytol 115:465–469

Clapperton MJ, Reid DM (1992) Effects of low-concentration sulphur dioxide fumigation and vesicular-arbuscular mycorrhizas on ^{14}C-partitioning in *Phleum pratense* L. New Phytol 120:381–387

Cooper KM (1984) Physiology of VA mycorrhizal associations. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhizae. CRC, Boca Raton, pp 155–203

Cox G, Sanders FE (1974) Ultrastructure of the host-fungus interface in a vesicular-arbuscular mycorrhiza. New Phytol 73:901–912

Cox G, Tinker PB (1976) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer: a quantitative ultrastructural study. New Phytol 77:371–378

Cox G, Sanders FE, Tinker PB, Wild JA (1975) Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London, pp 297–312

Crowe JH, Crowe LM, Chapman D (1984) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223:701–703

Dexheimer J, Gianinazzi S, Gianinazzi-Pearson V (1979) Ultrastructural cytochemistry of the host-fungus interface in the endomycorrhizal association *Glomus mosseae*/*Allium cepa*. Z Pflanzenphysiol 92:191–206

Dixon RK, Garrett HE, Bixby JA, Cox GS, Tompson JG (1981) Growth, ectomycorrhizal development and root soluble carbohydrates of black oak *Quercus velutina* seedlings fertilized by 2 methods. For Sci 27:617–624

Dixon RK, Garrett HE, Cox GS (1988) Carbohydrate relationships of *Citrus jambhiri* inoculated with *Glomus fasciculatum*. J Am Soc Hortic Sci 113:239–242

Dosskey MG, Linderman RG, Boersma L (1990) Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizae. New Phytol 115:269–274

Dosskey MG, Boersma L, Linderman RG (1991) Role for the photosynthate demand of ectomycorrhizas in the response of Douglas fir seedlings to drying soil. New Phytol 117:327–334

Douds DD Jr, Johnson CR, Koch KE (1988) Carbon cost of the fungal symbiont relative to the net leaf P accumulation in split-root VA mycorrhizal symbiosis. Plant Physiol 86:491–496

Eamus D, Jarvis PG (1989) The direct effects of increase in global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. Ad Ecol Res 19:1–55

Einig W, Hampp R (1990) Carbon partitioning in Norway spruce: amounts of fructose 2,6-bisphosphate and of intermediates of starch/sucrose synthesis in relation to needle age and degree of needle loss. Trees 4:9–15

Einig W, Wallander H, Hampp R, Nylund JE (1993) Carbohydrate status of needles of mycorrhizal and non-mycorrhizal Norway spruce seedlings under different nutrient regimes. Plant Physiol Suppl 102:177

Ekwebelam SA, Reid CPP (1983) Effect of light, nitrogen fertilization, and mycorrhizal fungi on growth and photosynthesis of lodgepole pine seedlings. Can J For Res 13:1099–1106

Erland S, Finlay R, Söderström B (1991) The influence of substrate pH on carbon translocation in ectomycorrhizal and non-mycorrhizal pine seedlings. New Phytol 119:235–242

Finlay R, Söderström B (1992) Mycorrhiza and carbon flow to the soil. In: Allen MJ (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, London, pp 134–160

Flügge UI, Heldt HW (1991) Metabolite translocators of the chloroplast envelope. Annu Rev Plant Physiol Plant Mol Biol 42:129–144

Geigenberger P, Stitt M (1991) Regulation of carbon partitioning between sucrose and nitrogen assimilation in cotyledons of germinating *Ricinus communis* L. seedlings. Planta 185:563–568

Gianinazzi-Pearson V, Gianinazzi S (1989) Cellular and genetical aspects of interactions between hosts and fungal symbionts in mycorrhizae. Genome 31:336–341

Giaquinta RT (1983) Phloem loading of sucrose. Annu Rev Plant Physiol 34:347–387

Giltrap NJ, Lewis DH (1981) Inhibition of growth of ectomycorrhizal fungi in culture by phosphate. New Phytol 87:669–675

Gorissen A, Joosten NN, Jansen AE (1991) Effects of ozone and ammonium sulphate on carbon partitioning to mycorrhizal roots of juvenile Douglas fir. New Phytol 119:243–250
Gould RP, Minchin PEH, Young PC (1988) The effects of sulphur dioxide on phloem transport in two cereals. J Exp Bot 39:997–1007
Guy CL (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu Rev Plant Physiol Plant Mol Biol 41:187–223
Hager A, Berthold W, Biber W, Edel HG, Lanz C, Schiebel G (1986) Primary and secondary energized ion translocating systems on membranes of plant cells. Ber D Bot Ges 99:281–295
Hampp R (1992) Comparative evaluation of the effects of gaseous pollutants, acidic deposition, and mineral deficiencies on the carbohydrate metabolism of trees. Agric, Ecosyst Environ 42:333–364
Hampp R, Schaeffer C, Wallenda T, Stülten C, Johann P, Einig W (1995) Changes in carbon partitioning or allocation due to ectomycorrhiza formation: biochemical evidence. Can J Bot 73(Suppl. 1):S548–S556
Harley JL (1991) The state of art. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology **23**. Academic Press, London, pp 1–23
Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London
Harris D, Paul EA (1987) Carbon requirements of vesicular-arbuscular mycorrhizae. In: Safir GR (ed) Ecophysiology of VA mycorrhizal plants. CRC, Boca Raton, pp 93–105
Harrison MJ (1996) A sugar transporter from *Medicago trunculata*: altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant J 9:491–503
Haselwandter K, Bobleter O, Read DJ (1990) Degradation of ^{14}C-labelled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. Arch Microbiol 153:352–354
Herold A (1980) Regulation of photosynthesis by sink activity – the missing link. New Phytol 86:131–144
Hers H-G, Van Schaftingen E (1982) Fructose 2,6-bisphosphate 2 years after its discovery. Biochemical Journal 206:1–12
Ho I, Trappe JM (1984) Effects of ozone exposure on mycorrhiza formation and growth of *Festuca arundinacea*. Environ Exp Bot 24:71–74
Hoffmann E, Wallenda T, Schaeffer C, Hampp R (1997) Cyclic AMP, a possible regulator of glycolysis in the ectomycorrhizal fungus *Amanita muscaria*. New Phytol 137:351–356
Hopf H, Kandler O (1976) Physiologie der Umbelliferose. Biochem Physiol Pflanz 169:5–36
Hult K, Gatenbeck S (1978) Production of NADPH in the mannitol cycle and its relation to polyketide formation in *Alternaria alternata*. Eur J Biochem 88:607–612
Hult K, Veide A, Gatenbeck S (1980) The distribution of the NADPH regenerating mannitol cycle among fungal species. Arch Microbiol 128:253–255
Ingestad T, Kähr M (1985) Nutrition and growth of coniferous seedlings at varied relative nitrogen addition rate. Physiol Plant 65:109–116
Ineichen K, Wiemken V, Wiemken A (1995) Shoots, roots and ectomycorrhiza formation of pine seedlings at elevated atmospheric carbon dioxide. Plant Cell Environm 18:703–707
Jakobsen I (1991) Carbon metabolism in mycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology **23**. Academic Press, London, pp 149–180
Jensen M, Feige GB, Waterkotte A (1991) Mannitol-1-phosphate dehydrogenases in *Pseudevernia furfuracea*. Lichenologist 34:187–196
Jennings DH (1995) The physiology of fungal nutrition. Cambridge University Press, Cambridge, UK

Jirjis R, Ramstedt M, Söderhäll K (1986) Mannitol does not inhibit glycolytic enzymes in roots of *Pinus sylvestris* and *Fagus orientalis*. New Phytol 102:285–291

Johnson CR, Graham JH, Leonard RT, Menge JA (1982a) Effect of flower bud development in *Chrysanthemum morifolium* on vesicular-arbuscular mycorrhiza formation. New Phytol 90:671–676

Johnson CR, Menge JA, Schwab S, Ting IP (1982b) Interaction of photoperiod and vesicular-arbuscular mycorrhizae on growth and metabolism of sweet orange *Citrus sinensis*. New Phytol 90:665–670

Kanazawa T, Kirk MR, Bassham JA (1970) Regulatory effects of ammonium on carbon metabolism in photosynthesising *Chlorella pyrenoidosa*. Biochim Biophys Acta 205:401–408

Kanazawa T, Kanazawa K, Kirk MR, Bassham JA (1972) Regulatory effects of ammonia on carbon metabolism in *Chlorella pyrenoidosa* during photosynthesis and respiration. Biochim Biophys Acta 256:656–669

Kendall EJ, Adams RP, Kartha KK (1990) Trehalase activity in plant tissue cultures. Phytochemistry 29:2525–2528

Kerr PS, Huber SC (1987) Coordinate control of sucrose formation in soybean leaves by sucrose-phosphate synthase and fructose-2,6-bisphosphate. Planta 170:197–204

Koch KE, Johnson CR (1984) Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root systems. Plant Physiol 75:26–30

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol 47:509–540

Koide RT, Schreiner RP (1992) Regulation of the vesicular-arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 43:557–581

Komor E (1983) Phloem loading and unloading. In: Esser K, Kubitzki K, Runge M, Schnepf E, Ziegler H (eds) Progress in botany, vol 45. Springer, Berlin Heidelberg New York, pp 68–75

Koziol MJ, Whatley FR, Shelvey JD (1988) An integrated view of the effects of gaseous air pollutants on plant carbohydrate metabolism. In: Schulte-Hostede S, Darrall NM, Blank LW, Wellburn AR (eds) Air pollution and plant metabolism. Elsevier, London, pp 148–168

Kozlowski TT (1992) Carbohydrate sources and sinks in woody plants. Bot Rev 58:107–222

Lamb RJ (1974) Effect of D-glucose on utilization of single carbon sources by ectomycorrhizal fungi. Trans Br Mycol Soc 63:295–306

Lapeyrie FF, Bruchet G (1985) Some factors influencing viability of ectomycorrhizal fungal inoculum. New Phytol 100:585–593

Larsen PO, Cornwell KL, Gee SL, Bassham JA (1981) Amino acid synthesis in photosynthesising spinach cells. Effects of ammonia on pool sizes and labelling from $^{14}CO_2$. Plant Physiol 68:292–299

Law R (1985) Evolution in a mutualistic environment. In: Boucher DH (ed) The biology of mutualism, ecology and evolution. Oxford University Press, New York, pp 145–170

Lei J, Dexheimer J (1988) Ultrastructural localization of ATPase activity in the *Pinus sylvestris*/*Laccaria laccata* ectomycorrhizal association. New Phytol 108:329–334

Lewis DH (1986) Interrelationships between carbon nutrition and morphogenesis in mycorrhizas. In: Gianinazzi-Pearson V, Gianinazzi S (eds). Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 85–100

Lewis DH, Harley JL (1965a) Carbohydrate physiology of mycorrhizal roots of beech. I. Identity of endogenous sugars and utilization of exogenous sugars. New Phytol 64:224–237

Lewis DH, Harley JL (1965b) Carbohydrate physiology of mycorrhizal roots of beech. II. Utilization of exogenous sugars by uninfected and mycorrhizal roots. New Phytol 64:238–255

Lewis DH, Harley JL (1965c) Carbohydrate physiology of mycorrhizal roots of beech. III. Movement of sugars between host and fungus. New Phytol 64:256–269
Lewis DH, Smith DC (1967) Sugar alcohols (polyols) in fungi and green plants. New Phytol 66:143–184
Lewis JD, Strain BR (1996) The role of mycorrhizas in the response of *Pinus taeda* seedlings to elevated CO_2. New Phytol 133:431–443
Lopez MF, Torrey JG (1985) Purification and properties of trehalase in *Frankia* ArI3. Arch Microbiol 143:209–215
Lösel DM, Cooper KM (1979) Incorporation of ^{14}C-labelled substrates by uninfected and VA mycorrhizal roots of onion. New Phytol 83:415–426
Mahoney MJ, Chevone BI, Skelly JM, Moore LD (1985) Influence of mycorrhizae on the growth of loblolly pine seedlings exposed to ozone and sulfur dioxide. Phytopathology 75:679–682
Mamoun M, Olivier JM (1991) Effect of carbon and nitrogen sources on *in vitro* growth of *Tuber melanosporum* Vitt. Application to mycelial biomass production. Agronomie 11:521–527
Martin F, Canet D, Marchal JP (1984) In vivo natural abundance carbon-^{13}NMR studies of the carbohydrate storage in ectomycorrhizal fungi. Physiol Veg 22:733–744
Martin F, Canet D, Marchal JP (1985) Carbon-^{13}NMR study of mannitol cycle and trehalose synthesis during glucose utilization by the ectomycorrhizal ascomycete *Cenococcum graniforme*. Plant Physiol 77:499–502
Martin F, Ramstedt M, Söderhäll K (1987) Carbon und nitrogen metabolism in ectomycorrhizal fungi and ectomycorrhizas. Biochimie 69:569–581
Martin F, Ramstedt M, Söderhäll K, Canet D (1988) Carbohydrate and amino acid metabolism in the ectomycorrhizal ascomycete *Sphaerosporella brunnea* during glucose utilization. A carbon-^{13}NMR study. Plant Physiol 86:935–940
Martin FM, Hilbert JL (1991) Morphological, biochemical and molecular changes during ectomycorrhiza development. Experientia 47:321–331
Marx C, Dexheimer J, Gianinazzi-Pearson V, Gianinazzi S (1982) Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. IV. Ultracytoenzymological evidence (ATPase) for active transfer processes in the host-arbuscular interface. New Phytol 90:37–43
McCool PM, Menge JA (1983) Influence of ozone on carbon partitioning in tomato. Potential role of carbon flow in regulation of the mycorrhizal symbiosis under conditions of stress. New Phytol 94:241–248
Meier S, Grand LF, Schoeneberger MM, Reinert RA, Bruck RI (1990) Growth, ectomycorrhizae and nonstructural carbohydrates of loblolly pine seedlings exposed to ozone and soil water deficit. Environ Pollut 64:11–28
Melin E, Nilsson H (1957) Transport of ^{14}C-labelled photosynthate to the fungal associate of pine mycorrhiza. Sven Bot Tidskr 51:166–186
Mellor RB (1992) Is trehalose a symbiotic determinant in symbioses between higher plants and microorganisms? Symbiosis 12:113–129
Meyer FH (1962) Die Buchen- und Fichtenmykorrhiza in verschiedenen Bodentypen, ihre Beeinflussung durch Mineraldüngung sowie für die Mykorrhizierung wichtige Faktoren. Mitt Bundesforschungsanstalt Forst-Holzwiss 54:1–73
Miller SL, Durall DM, Rygiewicz PT (1989) Temporal allocation of ^{14}C to extramatrical hyphae of ectomycorrhizal ponderosa pine seedlings. Tree Physiol 5:239–249
Mousseau M, Saugier B (1992) The direct effect of increased CO_2 on gas exchange and growth of forest tree species. J Exp Bot 43:1121–1130
Müller J, Staehelin C, Mellor RB, Boller T, Wiemken A (1992) Partial purification and characterization of trehalase from soybean nodules. J Plant Physiol 140:8–13

Namysl C, Rieger A, Hampp R, Dizengremel P (1991) Longitudinal distinction of adenine nucleotide pools in unmycorrhized and mycorrhized fine roots of spruce seedlings. Suppl Plant Physiol 96:1104

Nehls U, Wiese J, Guttenberger M, Hampp R (1998) Carbon allocation in ectomycorrhizas: Identification and expression analysis of an *Amanita muscaria* monosaccharide transporter. Molec Plant Microbe Interact 11:167–176

Nemec S, Guy G (1982) Carbohydrate status of mycorrhizal and nonmycorrhizal citrus rootstocks. J Am Soc Hortic Sci 107:177–180

Niederer M (1989) Ektomykorrihza in Bestandesfichten: die jahreszeitliche Dynamik löslicher Kohlenhydrate und ihre Bedeutung als Vitalitätsindikatoren. Ph D Thesis, University of Basel, Basel, Switzerland

Niederer M, Pankow W, Wiemken A (1989) Trehalose synthesis in mycorrhiza of Norway spruce. An indicator of vitality. Europ J For Pathol 19:14–20

Niederer M, Pankow W, Wiemken A (1992) Seasonal changes of soluble carbohydrates in mycorrhizas of Norway spruce and changes induced by exposure to frost and desiccation. Eur J For Pathol 22:291–299

Norby RJ, O'Neill EG, Luxmore RBJ (1986) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient poor soil. Plant Physiol 82:83–89

Norby RJ, O'Neill EG, Hood WG, Luxmore RBJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiol 3:203–210

Nylund JE (1988) The regulation of mycorrhiza formation – carbohydrate and hormone theories reviewed. Scand J For Res 3:465–479

Nylund JE, Unestam T (1987) Ectomycorrhiza in semi-hydroponic scots pine: increased photosynthesis but reduced growth. In: Sylvia DH, Hung LL, Graham JH (eds) Mycorrhizae in the next decade. Practical applications and research priorities. University of Florida, Gainesville

Nylund JE, Wallander H (1989) Effects of ectomycorrhiza on host growth and carbon balance in a semi-hydroponic cultivation system. New Phytol 112:389–398

Ocampo JA, Azcón R (1985) Relationship between the concentration of sugars in the roots and vesicular-arbuscular mycorrhizal infection. Plant Soil 86:95–100

Pacovsky RS (1989a) Carbohydrate, protein and amino acid status of *Glycine Glomus Bradyrhizobium* symbioses. Physiol Plant 75:346–354

Pacovsky RS (1989b) Metabolic differences in *Zea Glomus Azospirillum* symbioses. Soil Biol Biochem 21:953–960

Palmer JG, Hacskaylo E (1970) Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. Physiol Plant 23:1187–1197

Paul EA, Kucey RMN (1981) Carbon flow in plant microbial associations. Science 213:473–474

Pons S, Mudge KW, Negm FB (1986) Effect of mannitol on the *in vitro* growth, temperature optimum, and subsequent ectomycorrhizal infectivity of *Pisolithus tinctorius*. Can J Bot 64:1812–1816

Radin JW, Parker LL, Sell CR (1978) Partitioning of sugar between growth and nitrate reduction in cotton roots. Plant Physiol 62:550–553

Ramstedt M (1988) The significance of mannitol metabolism in ectomycorrhizal associations. Karstenia 28:61–62

Ramstedt M, Jirjis R, Söderhäll K (1987) Metabolism of mannitol in mycorrhizal and non-mycorrhizal fungi. New Phytol 105:281–288

Reich PB, Amundson RG (1985) Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566–570

Reich PB, Schoettle AW, Stroo HF, Troiano J, Amundson RG (1985) Effects of O_3, SO_2, and acidic rain on mycorrhizal infection in northern red oak seedlings. Can J Bot 63:2049–2055

Reid CPP, Kidd FA, Ekwebelam SA (1983) Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. Plant Soil 71:415–432

Rieger A, Guttenberger M, Hampp R (1992) Soluble carbohydrates in mycorrhized and non-mycorrhized fine roots of spruce seedlings. Z Naturforsch 47c:201–204

Robbins NS, Pharr DM (1988) Effect of restricted root growth on carbohydrate metabolism and whole plant growth of *Cucumis sativus* L. Plant Physiol 87:409–413

Saile P (1990) Charakterisierung der H^+-translocierenden ATPase am Plasmalemma von Ectomycorrhizapilzen und der Fichte unter Einbeziehung immunologischer Methoden. MSc. Thesis, University of Tübingen, Tübingen, Germany.

Salminen SO, Streeter JG (1986) Enzymes of alpha, alpha-trehalose metabolism in soybean nodules. Plant Physiol 81:583–541

Salzer P, Hager A (1991) Sucrose utilization of the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* depends on the cell wall-bound invertase activity of their host *Picea abies*. Bot Acta 104:439–445

Same BI, Robson AD, Abbott LK (1983) Phosphorus soluble carbohydrates and endomycorrhizal infection. Soil Biol Biochem 15:593–598

Schaeffer C, Wallenda T, Guttenberger M, Hampp R (1995) Acid invertase in mycorrhizal and non-mycorrhizal roots of Norway spruce (*Picea abies* [L.] Karst.) seedlings. New Phytol 129:417–424

Schaeffer C, Johann P, Nehls U, Hampp R (1996) Evidence for an up-regulation of the host and a down-regulation of the fungal phosphofructokinase activity in ectomycorrhizas of Norway spruce and fly agaric. New Phytol 134:697–702

Schiebel G (1988) Lokalisierung und Charakterisierung primär energetisierter H^+-Translokasen an Membranen von Ektomykorrhizapilzen (*Amanita muscaria* und *Hebeloma crustuliniforme*). Ph D Thesis, University of Tübingen, Tübingen, Germany

Schubert A, Wyss P, Wiemken A (1992) Occurence of trehalose in vesicular-arbuscular mycorrhizal fungi and in mycorrhizal roots. J Plant Physiol 140:41–45

Schwab SM, Menge JA, Tinker PB (1991) Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. New Phytol 117:387–398

Serrano R (1988) Structure and function of proton translocating ATPase in plasma membranes of plants and fungi. Biochim Biophys Acta 497:1–28

Shishkoff N (1987) Distribution of the dimorphic hypodermis of roots in angiosperm families. Ann Bot 60:1–15

Smith SE, Gianinazzi-Pearson V (1988) Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu Rev Plant Physiol Plant Mol Biol 39:221–244

Smith SE, Long CM, Smith FA (1989) Infection of roots with a dimorphic hypodermis: possible effects on solute uptake. In: Mejstrik V (ed) Ecological and applied aspects of ecto- and endomycorrhizal associations. Proc 2nd Eur Symp Mycorrhizae. Elsevier, Amsterdam, pp 403–407

Smith SE, Read DJ (1996) Mycorrhizal Symbiosis, 2nd edition, Academic Press, San Diego, ISBN 0-12-652840-3

Smith SE, Smith FA (1990) Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytol 114:1–38

Smith SE, St. John BJ, Smith FA, Nicholas DJD (1985) Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneum* L. and *Allium cepa* L.: effects of mycorrhizal infection and phosphate nutrition. New Phytol 99:211–227

Smith WH (1990) Air pollution and forests. Interaction between air contaminants and forest ecosystems, 2nd edn. Springer, Berlin Heidelberg New York, pp 313–345

Snellgrove RC, Splittstosser WE, Stribley DP, Tinker PB (1982) The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol 92:75–87

Söderström B, Finlay RD, Read DJ (1988) The structure and function of the vegetative mycelium of ectomycorrhizal plants. IV. Qualitative analysis of carbohydrate contents of mycelium interconnecting host plants. New Phytol 109:163–166

Stahl PD, Smith WK (1984) Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. Mycologia 76:261–267

Stitt M (1990) Fructose-2,6-bisphosphate as a regulatory molecule in plants. Annu Rev Plant Physiol Plant Mol Biol 41:153–185

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environm 14:741–762

Steingraber M, Outlaw WH Jr, Hampp R (1988) Subcellular compartmentation of fructose 2,6-bisphosphate in oat mesophyll cells. Planta 175:204–208

Stroo HF, Alexander M (1985) Effect of simulated acid rain on mycorrhizal infection of *Pinus strobus* L. Water Air Soil Pollut 25:107–114

Stroo HF, Reich PB, Schoettle AW, Amundson RG (1988) Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. II. Mycorrhizal infection. Can J Bot 66:1510–1516

Taber WA, Taber RA (1987) Carbon nutrition and respiration of *Pisolithus tinctorius*. Trans Br Mycol Soc 89:13–26

Tester M, Smith FA, Smith SE (1985) Phosphate inflow into *Trifolium subterraneum*. Effects of photon irradiance and mycorrhizal infection. Soil Biol Biochem 17:807–810

Theodorou C, Reddell P (1991) In vitro synthesis of ectomycorrhizas on Casuarinaceae with a range of mycorrhizal fungi. New Phytol 118:279–288

Thevelein JM (1984) Regulation of trehalose mobilization in fungi. Microbiol Rev 48:42–59

Thomson BD, Robson AD, Abbott LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora caulospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol 103:751–766

Thomson BD, Robson AD, Abbott LK (1990) Mycorrhizas formed by *Gigaspora caulospora* and *Glomus fasciculatum* on subterranean clover in relation to soluble carbohydrate concentrations in roots. New Phytol 114:217–225

Tingey DT, Andersen CP (1991) The physiological basis of differential plant sensitivity to changes in atmospheric quality. In: Taylor GE Jr, Pitelka LF, Clegg MT (eds) Ecological genetics and air pollution. Springer, Berlin Heidelberg New York, pp 209–234

Turpin DH, Botha FC, Smith RG, Feil R, Horsey AK, Vandenbergh GC (1990) Regulation of carbon partitioning to respiration during dark ammonium assimilation by the green alga *Selenastrum minutum*. Plant Physiol 93:166–175

Veluthambi K, Mahadevan S, Maheshwari R (1981) Trehalose toxicity in *Cuscuta reflexa*: correlation with low trehalase activity. Plant Physiol 68:1369–1374

Wallander H (1992) Regulation of ectomycorrhizal symbiosis in *Pinus sylvestris* L. seedlings. Influence of mineral nutrition. Ph D Thesis, Swedish University of Agricultural Sciences, Department of Forest Mycology and Pathology, Uppsala, Sweden

Wallander H (1995) A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. Plant Soil 168/169:243–248

Wallenda T (1996) Untersuchungen zur Physiologie der Pilzpartner von Ektomykorrhizen der Fichte (*Picea abies* [L.] Karst.). PhD Thesis, Faculty for Biology, University of Tübingen, Germany

Wallander H, Nylund JE (1991) Effects of excess nitrogen on carbohydrate concentration and mycorrhizal development of *Pinus sylvestris* L. seedlings. New Phytol 119:405–411

Wallenda T, Schaeffer C, Einig W, Wingler A, Hampp R, Seith B, George E, Marschner H (1996) Effects of varied soil nitrogen supply on Norway spruce (*Picea abies* [L.] Karst.) II. Carbon metabolism in needles and mycorrhizal roots. Plant Soil 186:361–369

Wang GM, Colemann DC, Freckman DW, Dyer MI, McNaughton SJ, Acra MA, Goeschl JD (1989) Carbon partitioning patterns of mycorrhizal versus non-mycorrhizal plants: real-time dynamic measurements using $^{11}CO_2$. New Phytol 112:489–493

Wedding RT, Harley JL (1976) Fungal polyol metabolites in the control of carbohydrate metabolism of mycorrhizal roots of beech. New Phytol 77:675–688

Wieser U, Pankow W, Wiemken A (1986) The adenylate energy charge of vesicular-arbuscular mycorrhiza of onion (*Allium cepa* L.). J Plant Physiol 124:181–186

Williams PG (1992) Axenic culture of arbuscular mycorrhizal fungi. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology 24. Academic Press, London, pp 203–220

Wingler A (1992) Untersuchungen zum Mannit-Stoffwechsel des Ektomykorrhiza-Systems Fichte (*Picea abies* [L.] Karst.) / Fliegenpilz (*Amanita muscaria* [L. ex FR.] Hooker). MSc Thesis, University of Tübingen, Tübingen, Germany

Wingler A, Wallenda T, Hampp R (1996) Mycorrhiza formation on Norway spruce (*Picea abies*) roots affects the pathway of anaplerotic CO_2 fixation. Physiol Plant 96:699–705

Ziegler H (1975) Phloem transport. Nature of transported substances. In: Zimmermann MH, Milburn JA (eds) Encyclopedia of plant physiology, New Series, vol 1. Transport in plants. Springer, Berlin Heidelberg New York, pp 59–100